Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

THE ANAPHYLACTOGENIC ACTIVITY OF SOME VEGETABLE PROTEINS.*†

THE BIOLOGICAL REACTIONS OF THE VEGETABLE PROTEINS. V.

H. GIDEON WELLS AND THOMAS B. OSBORNE.

(From the Pathological Laboratory of the University of Chicago and the Connecticut Agricultural Experiment Station, New Haven, Connecticut.)

In an earlier paper[1] we gave the results of anaphylaxis experiments in which carefully purified preparations of a large number of typical vegetable proteins were used. These preparations represent the principal part of the reserve protein of the seeds from which each had been obtained, and differ in their solubility and chemical constitution to a marked extent from the protein constituents of the sera and other fluids of animal origin, with which most anaphylaxis experiments had previously been made. In the course of our investigation it was found that while the minimum sensitizing dose of edestin from the hemp-seed, *Cannabis sativa*, was practically the same as that of the globulin from the squash-seed, *Cucurbita maxima*, and likewise that the minimum quantities of these proteins required to produce anaphylactic intoxication were also about the same, great differences in activity existed between intoxicating doses which were larger than the minimum. Thus, of 19 sensitized guinea-pigs injected with 100-mg. doses of squash-seed globulin 18 died of anaphylactic shock, whereas in 15 similar experiments with edestin but 2 fatal reactions were obtained.

This fact led us to suggest that the difference might be due to differences in the solubility of these proteins in the body fluids. This possibility was supported by the fact that, in the peritoneal cavity, edestin is precipitated from the dilute alkalin solutions injected, and consequently may not be redissolved and carried to the blood quickly enough to cause severe intoxication, where, according to White and Avery,[2] 0.5 mg. edestin gives fatal reactions.

---

* Received for publication November 24, 1913.

† A part of the expense of this investigation was shared by the Carnegie Institution of Washington

[1] *Jour. Infect. Dis.*, 1911, 8, p. 106.

[2] *Ibid.*, 1913, 13, p. 103.

If such were the case the severity of the symptoms would be limited by the solubility of the protein in the peritoneal fluids rather than by the amount injected. None of the vegetable proteins which we then used possessed the high solubility in dilute saline solutions which characterized the chief protein constituents of such animal fluids as had previously been used in anaphylaxis experiments, and which had been shown to produce anaphylactic intoxication in much smaller doses.

We have now made a series of experiments under uniform conditions in order to obtain more extensive data concerning the relative anaphylactic toxicity of several of these reserve proteins of seeds, and have extended their scope to include preparations of the so-called *proteoses* from the Brazil-nut, *Bertholletia excelsa,* and the Soy-bean, *Glycine soja.* These preparations represent the part of the protein which is not precipitated from the sodium chlorid extract of this seed after prolonged dialyzing and subsequently heating the filtered solution to 100° C. They were separated from their concentrated aqueous solutions by adding alcohol, and purified by reprecipitating. These preparations contain all of the protein remaining in the extract after separating the globulins and albumins, and may consist of one or several individual protein substances. Nothing definite is known as to the chemical constitution of these proteoses, nor their relations to proteoses obtained by the action of proteolytic enzymes. They are called proteoses simply because they resemble this class of proteins in solubility. Whether they exist preformed in the seed, or are formed from the reserve protein of the seed by the action of the seed enzymes during the process of extraction, etc., is not known. (The biological reactions of the vegetable "proteoses" will be discussed in a forthcoming paper.)

The important point in connection with these present experiments is simply that these preparations from the Brazil-nut and Soy-bean represent a type of vegetable protein which is highly soluble in water and is precipitated from its solutions with great difficulty.

The results of our new experiments are given in Table 1. The terms used in describing the severity of the reactions are applied

TABLE 1.

MINIMUM INTOXICATING DOSE OF SOME VEGETABLE PROTEINS WHEN INJECTED INTRAPERITONEALLY INTO GUINEA-PIGS SENSITIZED 3 WEEKS PREVIOUSLY WITH 1 MG. OF THE SAME PROTEIN.

	0.05 mg.	0.1 mg.	0.5 mg.	1 mg.	2 mg.	3 mg.	5 mg.	10 mg.	20 mg.	40 mg.
1. Castor-bean globulin (Ricinus communis)	....	....	Slight	Doubtful Moderate	Slight	Slight Doubtful Moderate	Slight	Severe	....	....
2. Squash-seed globulin (Cucurbita maxima)	....	....	0	Slight Doubtful	....	Slight	Moderate Moderate	Severe	Severe	....
3. Edestin (Cannabis sativa)	....	....	0	Doubtful	Slight	....	Slight	Moderate	Severe	Severe
4. Excelsin (Bertholletia excelsa)	....	....	0	Doubtful Slight	Moderate	....	Moderate	Moderate	Fatal	....
5. Pea legumin (Pisum sativum)	....	....	0	Slight 0	Doubtful 0	....	Slight Moderate	Slight Doubtful Doubtful	Moderate	....
6. Vicilin (Pisum sativum)	....	....	0	Doubtful Doubtful Severe	Slight	....	Slight Severe Doubtful	Slight	Slight	Moderate
7. Vignin (Vigna sinensis)	....	....	0	Slight	Moderate	....	Severe Moderate	Fatal Fatal	Fatal	....
8. Glycinin (Soja hispida)	....	....	Doubtful	Slight	Slight	....	Slight	Moderate	Severe Slight	....
9. Wheat gliadin (Triticum vulgare)	....	....	Doubtful 0 Severe	Slight Moderate	Severe	....	Fatal	Severe Doubtful	....	....
10. Hordein (Hordeum vulgare)	....	....	Slight Moderate	Slight Moderate	Slight Moderate	Moderate	Moderate Fatal	Severe Severe	....	....
11. Zein (Zea mays)	....	....	0	Slight Doubtful	Slight	....	Moderate	Moderate	....	....
12. Brazil-nut proteose (Bertholletia excelsa)	Slight	Severe	Fatal 68 min. Fatal 80 min.	Fatal 40 min.	Fatal 38 min.	....	....	....	....	....
13. Soy-bean proteose (Glycine soja)	Moderate Doubtful with 0.01 mg.	Moderate Moderate	Moderate	Severe	Fatal 2 hrs.	....	....	....	....	....

with the same significance as in all our previous articles, and are defined in the first paper.[1]

The eight proteins appearing first in this table all belong to the group of globulins; that is, they are insoluble in neutral aqueous solutions but soluble in neutral saline solutions. None of them, except vicilin, dissolves to any marked extent in such dilute saline solutions as do the globulins present in most animal fluids. They are, therefore, probably absorbed from the peritoneal cavity relatively slowly.

Gliadin and hordein represent a totally different type of solubility, being practically insoluble in dilute neutral saline solutions and but slightly soluble in pure water. Their most marked characteristic is their solubility in alcohol of 80–90 per cent. Zein belongs to the same class, but is insoluble in water or saline solutions and very readily soluble in alcohol of 90–95 per cent.

The solubility of the proteoses has already been described.

It is difficult to interpret accurately the results given in the table according to the relative solubility of these proteins, for with the exception of the proteoses, the degree of solubility of each when injected into the peritoneum will depend on factors of which at present we have but little knowledge. Thus while edestin is insoluble in pure water it is readily dissolved by water containing minute amounts of acids or alkalies. The addition of a very small quantity of a neutral inorganic salt at once precipitates the salt of edestin formed by its union with the added acid or base, while a larger quantity of the same neutral salt will redissolve the precipitate. The proportion of inorganic salt thus causing precipitation, or resolution, depends on the proportion of acid or alkali combined with the edestin. In view of such intricate solubility relations it is evident that with our present knowledge it is impossible to draw definite conclusions respecting the amount of injected protein which may be precipitated in the peritoneal cavity.

The results given in the table, however, are in the main in harmony with what is at present known concerning the relative ease with which the protein might be expected to be precipitated from the dilute alkalin solutions used for intoxication. Thus, with the exception of edestin and vicilin, toxic symptoms begin to appear when one milligram of the globulin is used, while with larger quantities the results obtained with these several globulins do not differ from one another very greatly. Edestin is the least toxic of this series, and from such comparisons of its solubility with that of the other globulins, as have yet been made, it seems probable that it is more easily precipitated in the peritoneal cavity than they are. Vicilin appears to be the most toxic of this series of globulins, which is in harmony with the fact that it dissolves abundantly in much more dilute saline solutions than do any of these other globulins.

---

[1] *Loc. cit.*

The alcohol-soluble proteins, gliadin and hordein, are about equally toxic, and more so than any of the other proteins in this list except the proteoses. Whether this is due to their peculiar chemical constitution, or to their more ready solubility in the body fluids, cannot be definitely determined from available data. That the latter is the more probable explanation is indicated by the fact that zein, which is much more easily precipitated from dilute alkalin solutions than are gliadin or hordein, is distinctly less toxic anaphylactically. The strongest evidence that the degree of toxicity is determined by the solubility of the injected protein is shown by the experiments with the proteoses from the Soy-bean and the Brazil-nut, for the minimal lethal dose of these preparations is far smaller than that of any of the other vegetable proteins tested, and is of the same order of magnitude as that of egg albumin or of serum proteins.

It is impossible to reproduce accurately the conditions that obtain within the animal body, and thus determine the exact degree of solubility of our vegetable proteins in either the peritoneal fluids or the blood. The best we can do is to determine their precipitability by serum, and experiments of this sort indicate that there is a very close correspondence between the precipitability of the vegetable proteins and their toxicity for sensitized animals. Such experiments were conducted as follows:

To small test tubes, each containing 0.5 c.c. of normal beef serum, kept sterile for some time with chloroform, which was removed before using, was carefully added 0.1 c.c. of the solution of the protein to be tested, dissolved in 0.1 per cent NaOH, which was the solvent usually employed in our animal experiments. The solution was run upon the surface of the serum with a minimum of admixture, and the tubes stoppered. After the solution had stood for one hour at room temperature the amount of turbidity or precipitate at the line of contact was noted and indicated in the table as "zone," the standard for comparison being a set of tubes prepared at the same time, and in the same proportions, with edestin. The tubes were then thoroughly shaken and left in the icebox for 18 hrs., when the amount of sediment was again observed and compared with that in the tube to which edestin had been added, being indicated in the table as "precipitate." Since the serum was always more or less opalescent or turbid, as were also many of the protein solutions, the reactions given in the following table as "traces" or "doubtful" have little or no significance.

TABLE 2.

PRECIPITATION OF VEGETABLE PROTEINS BY SERUM.

Strength of Protein Solution	1 Per Cent	0.2 Per Cent	0.1 Per Cent	0.05 Per Cent	0.01 Per Cent	0.005 Per Cent	Percentage of Fatal or Severe Reactions with 100 mg. Doses
Edestin: zone	+++++	+++	++	++	+	+	33
precipitate	+++++	++	+	+	Tr.	o	
Zein: zone	++++	++	+	Tr.	d	d	30
precipitate	+++++	+	Tr.	Tr.	o	o	
Gliadin (wheat): zone	+++	++	+	Tr.	d	o	67
precipitate	++	+	Tr.	o	o	o	
Hordein: zone	+++	++	+	Tr.	d	d	69
precipitate	++	+	+	Tr.	o	o	
Excelsin: zone	++	+	+	+	Tr.	Tr.	94
precipitate	+++	o	o	o	o	o	
Squash-seed globulin: zone	++	Tr.	Tr.	o	o	o	95
precipitate	+++	Tr.	o	d	d	o	
Flax-seed globulin: zone	+	+	Tr.	d	d	o	....
precipitate	+	o	o	o	o	o	
Vignin: zone	Tr.	o	o	o	o	o	100
precipitate	+	o	o	o	o	o	
Proteose, excelsin: zone	Tr.	o	o	o	o	o	100
precipitate	d	o	d	o	o	o	
Proteose, Soy-bean: zone	Tr.	o	o	o	o	o	100
precipitate	o	o	o	o	o	o	

Arranging the preparations in the apparent order of precipitation, we find that edestin and zein, which least often produce severe or fatal intoxications on intraperitoneal injection into sensitized guinea-pigs, give by far the greatest amount of precipitate with serum. At the other end of the list come the vegetable "proteoses," such as Brazil-nut proteose and Soy-bean proteose, which we have found to be quite as toxic (anaphylactically) as serum or egg-white proteins, which likewise give little, if any, precipitate with beef serum under the conditions of the experiment. Flax-seed globulin, squash-seed globulin, vignin, and excelsin, which are somewhat less active, produce more precipitate than the "proteoses," but less than hordein and gliadin which they exceed in anaphylactic toxicity. Doses of 50–100 mg. of gliadin or hordein give fatal or severe reactions with sensitized guinea-pigs less frequently than do flax-seed globulin, squash-seed globulin, excelsin, or vignin. Thus, in an earlier series of experiments, the percentage of fatal and severe reactions with 100-mg. doses of these proteins was as follows: squash-seed globulin, 95; vignin, 100; excelsin, 94; hordein, 69; gliadin, 67; zein, 33; edestin, 30. The percentage of fatal reactions was: squash-seed globulin, 95; vignin, 72; excelsin, 67; hordein, 44; gliadin, 17; edestin, 13; zein, 0.

On the other hand, in the series of experiments recorded in this paper, 5 mg. of gliadin or hordein, 10 mg. of vignin, and 20 mg. of excelsin were the smallest quantities, respectively, causing death. With the other proteins even in doses of 20 mg. no fatal reactions were obtained. We thus see that the minimum intoxicating doses of these proteins bear no exact relation to their relative toxicity when given in larger doses; but it is of significance that the, comparatively speaking, extremely soluble "proteoses" are toxic in the smallest doses and also almost always produce fatal reactions in sensitized animals.

The efficiency of these vegetable proteins in producing severe intoxication in sensitized animals, therefore, corresponds in a general way to their precipitability by serum as thus determined, and presumably depends upon the extent to which they are dissolved and absorbed into the blood after injection into the peritoneal cavity.

CONCLUSIONS.

The data presented in this paper support the assumption that the severity of the anaphylaxis reactions produced by intraperitoneal injections of dilute, alkalin solutions of vegetable proteins is, approximately, in inverse ratio to their relative precipitability when their solutions are mixed with the peritoneal fluid.

Proteins like edestin, which are readily precipitated, and only slowly redissolved, under conditions similar to those presumably prevailing in the peritoneum, rarely produce a fatal intoxication;

whereas those that are less easily precipitated, and more readily dissolved, give fatal reactions in much smaller doses.

The lethal dose of those vegetable proteins which are readily soluble in pure water, or in very dilute saline solutions, is smaller than that of those which are soluble only in comparatively strong saline solutions.

The minimum intoxicating dose of most of the vegetable globulins (i.e., proteins insoluble in pure water but soluble in saline solutions) administered intraperitoneally is from 1 to 2 mg., but from 5 to 10 mg. are usually required for severe intoxication.

On the other hand, the minimum intoxicating dose of those so-called vegetable proteoses which are readily soluble in pure water and are uncoagulable by heat is much smaller, moderate to severe reactions being obtained with 0.05–0.1 mg. and fatal results with 0.5–2 mg.